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Synthesis and bioactivity evaluation of new pyrimidinone-5-carbonitriles as potential anticancer and antimicrobial agents

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Abstract New series of pyrimidinone-5-carbonitriles **3a–i**, **4a–e**, **5a–c**, **6** and **7** have been synthesized and explored for their activities as anticancer, antibacterial and antifungal agents. Investigation of the anticancer activity revealed that several newly synthesized derivatives displayed potent cytotoxic activity against different cancer cells. Among them, compound **3g** was the most potent on the MCF-7, A549 and Caco-2 cell lines (IC_{50} = 1.42, 1.98 and 9.50 μM, respectively), as compared with 5-fluorouracil (IC_{50} = 1.71, 10.32 and 20.22 μM, respectively), while compound **3f** was found especially effective against MCF-7 and Caco-2 cell lines (IC_{50} = 1.48 and 16.15 μM, respectively). Furthermore, the antimicrobial evaluation showed that compounds **3f** and **3g** have potent antibacterial activity against Gram-positive bacteria *Staphylococcus aureus* (MIC = 4 and 8 μg/mL, respectively) and promising activity against *Escherichia coli* (IZ. = 19 and 17 mm, respectively). Meanwhile, compound **4b** displayed the highest activity toward *Bacillus subtilis* (MIC = 8 μg/mL). In particular, the results suggested that hydrazone derivatives

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bearing heterocyclic rings **3f** and **3g** are good lead compounds for the future design of more potent anticancer or antimicrobial agents.

Keywords Anticancer · Antibacterial · Antifungal · Pyrimidinone-5-carbonotrile

Introduction

Pyrimidines are considered to be an important chemical synthon of various therapeutic efficacies and pharmaceutical utility. They possess diverse pharmacological activities, the most pronounced of which are anticancer [1–9] and antimicrobial [10–14]. Furthermore, various drugs containing a pyrimidine nucleus are in clinical use as anticancer agents, for example, 5-fluorouracil (5-FU), tegafur and imatinib (Gleevec™; Fig. 1) [15, 16]. In addition, pyrimidinone-5-carbonitrile derivatives **I–III** were reported as anticancer and antimicrobial agents (Fig. 1) [17–19]. Moreover, a literature survey revealed that elaborating the pyrimidinone-5-carboitrile scaffold with a hydrazinyl, hydrazide or hydazone moiety in compounds **IV–VI** conferred promising chemotherapeutic activity as anticancer and antimicrobial agents (Fig. 1) [20, 21].

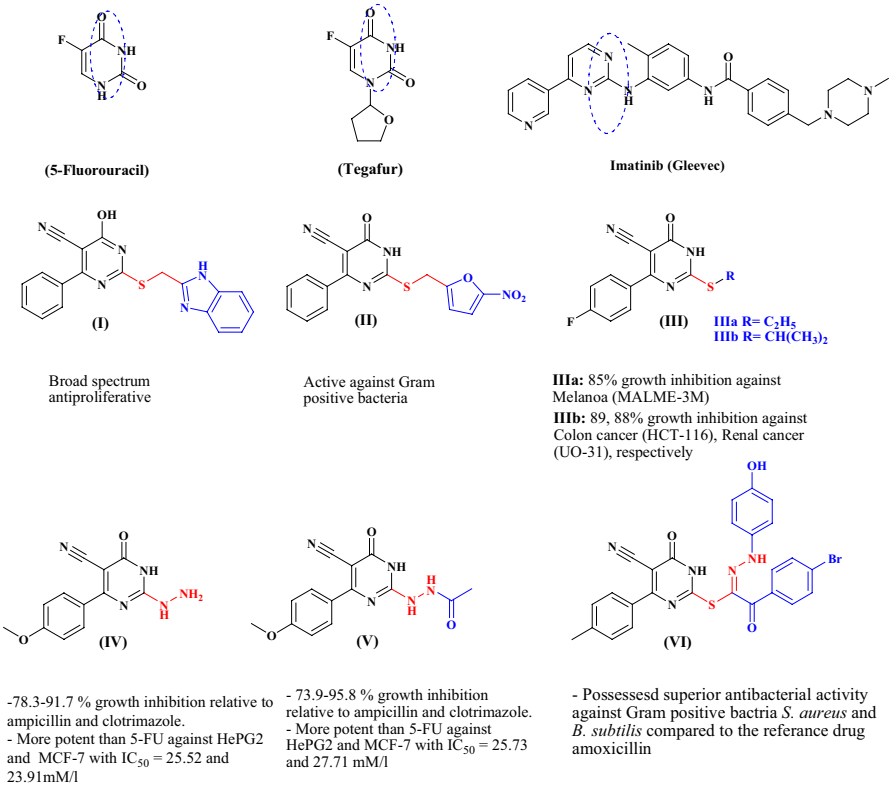


Fig. 1 Pyrimidine derivatives as anticancer and antimicrobial agents

In view of the biological significance of the pyrimidinone-5-carbonitriles, we herein report the synthesis and biological evaluation of novel pyrimidinone-5-carbonitrile derivatives **3a–i**, **4a–e**, **5a–c**, **6** and **7** as anticancer and antimicrobial agents. The design of the target compounds was initiated through structural extension of the lead compound **2** identified in our lab [19] by different arylidine groups to obtain the hydrazone derivatives **3a–i** with an active azomethine (N=CH) proton. The azomethine group is well-acknowledged to be the antitumor and antimicrobial pharmacophore for a plethora of hydrazone derivatives [22]. Furthermore, two other series of pyrimidinone-5-carbonitriles were prepared in which modification was focused on changing the methine spacer in **3a–i** by C=O in **4a–e** or CH_2–C=O in **5a–c** to study the effect of such structural modifications on the desired biological activities. Finally, utilizing the hydrazinyl group in **2** for incorporation of a pharmacophoric ring, (pyrazol-1-yl)pyrimidinone **6** and (pyrazolidin-1-yl)pyrimidinone **7** were prepared (Fig. 2).

Experimental part

General

All reagents were commercially available and were used without further purification. Melting points were determined on a Stuart apparatus and the values given are

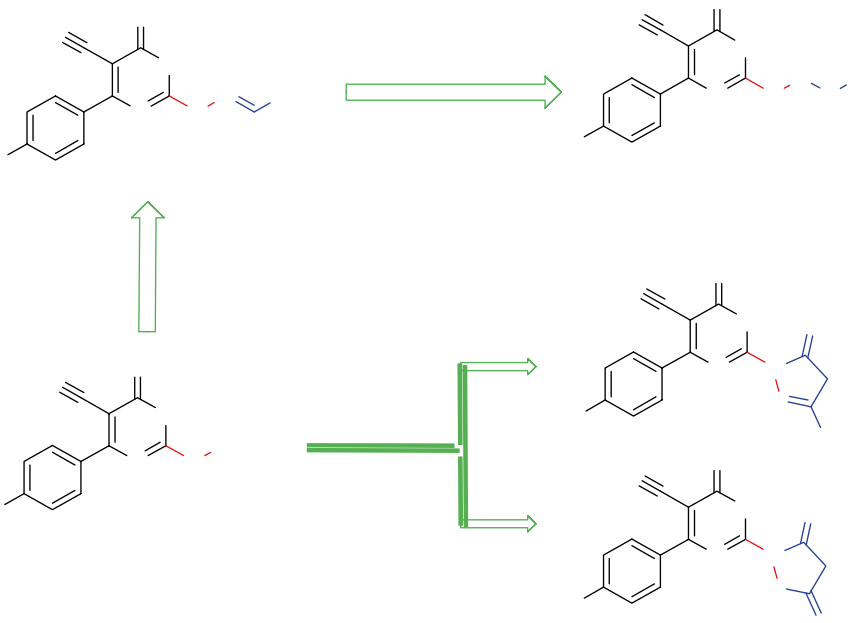


Fig. 2 Design of the target compounds **3a–i**, **4a–e**, **5a–c**, **6** and **7**

uncorrected. IR spectra were determined as KBr discs on a Shimadzu IR 435 spectrophotometer (Faculty of Pharmacy, Cairo University, Egypt) and the values are represented in cm^{-1}.^{1}H-NMR spectra were recorded on Varian Gemini 300-MHz and 400-MHz spectrophotometers using TMS as an internal standard, and chemical shift values were recorded in ppm on a δ scale (Microanalytical Center, Cairo University, Egypt). ^{13}C-NMR spectra were recorded on Varian Gemini 75 MHz spectrophotometer using TMS as internal standard and chemical shift values were recorded in ppm on a δ scale (Microanalytical Center, Cairo University, Egypt). Mass spectra were recorded on a Hewlett Packard 5988 spectrometer (Microanalytical Center, Cairo University, Egypt). Elemental analyses were carried out at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Egypt. Progress of the reactions was monitored using TLC aluminum sheets precoated with UV fluorescent silica gel (Merck 60F 254) and were visualized using a UV lamp.

6-(4-Fluorophenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**1**) and 4-(4-fluorophenyl)-2-hydrazinyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**2**) were synthesized according to the reported procedures [19, 23].

General procedure of synthesis of compounds (3a–i)

A mixture of compound **2** (0.5 g, 2 mmol), the appropriate aldehyde (2 mmol) and glacial acetic acid (1 mL) in absolute ethanol (20 mL) was heated under reflux for 6 h. The obtained precipitate was filtered, dried and crystallized from ethanol.

*2-(2-(4-Fluorobenzylidene)hydrazinyl)-4-(4-fluorophenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**3a**)*

Dark yellow crystals; (0.35 g, 1.00 mmol), yield: 49.78%; m.p.: 320–322 °C; IR (cm^{-1}): 3273 (2NH), 3140 (N=CH + ArH), 2218 (C≡N), 1640 (C=O); ^{1}H-NMR (DMSO-d_6-300 MHz, ppm): 6.80 (d, 2H, J = 9.0 Hz, Ar<u>H</u>), 7.10–7.29 (m, 2H, Ar<u>H</u>), 7.52 (d, 2H, J = 9.0 Hz, Ar<u>H</u>), 7.72–8.08 (m, 2H, Ar<u>H</u>), 8.17 (s, 1H, N=C<u>H</u>), 9.76 (s, 1H, N<u>H</u>, exchangeable by D_2O), 10.58 (s, 1H, N<u>H</u>, exchangeable by D_2O); Anal. calcd. for $C_{18}H_{11}F_2N_5O$ (351.31): C, 61.54; H, 3.16; N, 19.93. Found: C, 61.62; H, 3.22; N, 20.05.

*2-(2-(4-Chlorobenzylidene)hydrazinyl)-4-(4-fluorophenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**3b**)*

Yellow crystals; (0.48 g, 1.31 mmol), yield: 64.86%; m.p.: 318–320 °C; IR (cm^{-1}): 3367 (2NH), 3138 (N=CH + ArH), 2216 (C≡N), 1666 (C=O); ^{1}H-NMR (DMSO-d_6-300 MHz, ppm): 7.35–7.38 (m, 2H, Ar<u>H</u>), 7.50 (d, 2H, J = 8.7 Hz, Ar<u>H</u>), 7.91–7.95 (m, 2H, Ar<u>H</u>), 8.06 (d, 2H, J = 8.7 Hz, Ar<u>H</u>), 8.17 (s, 1H, N=C<u>H</u>), 12.50 (s, 2H, 2N<u>H</u>, exchangeable by D_2O); Anal. calcd. for $C_{18}H_{11}ClFN_5O$ (367.76): C, 58.79; H, 3.01; N, 19.04. Found: C, 58.87; H, 3.08; N, 19.18.

*4-(4-Fluorophenyl)-2-(2-(4-methylbenzylidene)hydrazinyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**3c**)*

Orange crystals; (0.21 g, 0.605 mmol), yield: 30.25%; m.p.: 296–298 °C; IR (cm^{-1}): 3369, 3261 (2NH), 3159 (N=CH + ArH), 2850 (CH aliphatic), 2214 (C≡N), 1664 (C=O); ^{1}H-NMR (DMSO-d_6-300 MHz, ppm): 2.34 (s, 3H, C$\underline{H}_3$), 7.15 (d, 2H, J = 9.0 Hz, Ar$\underline{H}$), 7.21–7.26 (m, 2H, Ar$\underline{H}$), 7.59 (d, 2H, J = 9.0 Hz, Ar$\underline{H}$), 7.89–7.94 (m, 2H, Ar$\underline{H}$), 8.15 (s, 1H, N=C$\underline{H}$), 10.75 (s, 1H, N$\underline{H}$, exchangeable by D_2O), 12.15 (s, 1H, N$\underline{H}$ exchangeable by D_2O);^{13}C-NMR (DMSO-d_6-75 MHz, ppm): 20.96, 83.86, 111.01, 117.92, 125.40, 125.98, 127.91, 129.24, 130.18, 131.02, 132.51,138.10, 139.15, 140.14, 146.64, 147.92, 152.86, 162.04, 169.20; MS (*m/z*): 347.00 (M^+, 18.39%), 348.00 (M + H, 18.71%), 349.00 (M + 2, 12.26%), 103.00 (100.00%); Anal. calcd. for $C_{19}H_{14}FN_5O$ (347.35): C, 65.70; H, 4.06; N, 20.16. Found: C, 65.81; H, 4.13; N, 20.24.

*4-(4-Fluorophenyl)-2-(2-(4-methoxybenzylidene)hydrazinyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**3d**)*

Dark yellow crystals; (0.44 g, 1.21 mmol), yield: 60.52%; m.p.: 204–206 °C; IR (cm^{-1}): 3360, 3311 (2NH), 3134 (N=CH + ArH), 2830 (CH aliphatic), 2206 (C≡N), 1662 (C=O); ^{1}H-NMR (DMSO-d_6-300 MHz, ppm): 3.79 (s, 3H, OC$\underline{H}_3$), 6.98 (d, 2H, J = 8.7 Hz, Ar$\underline{H}$), 7.09–7.15 (m, 2H, Ar$\underline{H}$), 7.64 (d, 2H, J = 8.7 Hz, Ar$\underline{H}$), 7.89–7.98 (m, 2H, Ar$\underline{H}$), 8.13 (s, 1H, N=C$\underline{H}$), 10.60 (s, 1H, N$\underline{H}$, exchangeable by D_2O), 10.66 (s, 1H, N$\underline{H}$, exchangeable by D_2O); MS (*m/z*): 363.10 (M^+, 1.90%), 364.10 (M + H, 2.39%), 135.05 (100%); Anal. calcd. for $C_{19}H_{14}FN_5O_2$ (363.35): C, 62.81; H, 3.88; N, 19.27. Found: C, 62.89; H, 3.87; N, 19.42.

*4-(4-Fluorophenyl)-2-(2-(3,4,5-trimethoxybenzylidene)hydrazinyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**3e**)*

Yellow crystals; (0.64 g, 1.51 mmol), yield: 75.29%; m.p.: 256–258 °C; IR (cm^{-1}): 3358, 3328 (2NH), 3120 (N=CH + ArH), 2939 (CH aliphatic), 2204 (C≡N), 1660 (C=O); ^{1}H-NMR (DMSO-d_6-300 MHz, ppm): 3.70 (s, 3H, OC$\underline{H}_3$), 3.87 (s, 6H, 2(OCH_3)) 7.01 (s, 2H, Ar$\underline{H}$), 7.15 (d, 2H, J = 9.0 Hz, Ar$\underline{H}$), 7.82 (s, 1H, N$\underline{H}$, exchangeable by D_2O), 7.84 (d, 2H, J = 9.0 Hz, Ar$\underline{H}$), 8.16 (s, 1H, N = C$\underline{H}$), 10.74 (s, 1H, N$\underline{H}$, exchangeable by D_2O); Anal. calcd. for $C_{21}H_{18}FN_5O_4$ (423.40): C, 59.57; H, 4.29; N, 16.54. Found: C, 59.61; H, 4.35; N, 16.69.

*4-(4-Fluorophenyl)-2-(2-((5-methylfuran-2-yl)methylene)hydrazinyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**3f**)*

Brown red crystals; (0.41 g, 1.21 mmol), yield: 60.74%; m.p.: 230–232 °C; IR (cm^{-1}): 3323 (2NH), 3101 (N=CH + ArH), 2879 (CH aliphatic) 2208 (C≡N), 1660 (C=O); ^{1}H-NMR (DMSO-d_6-300 MHz, ppm): 2.34 (s, 3H, CH_3), 6.77 (d, 1H, Ar$\underline{H}$), 6.80 (d, 1H,Ar$\underline{H}$), 7.75–7.82 (m, 4H, Ar$\underline{H}$), 8.10 (s, 1H, N=C$\underline{H}$), 10.20 (s, 1H, N$\underline{H}$, exchangeable by D_2O), 10.66 (s, 1H, N$\underline{H}$, exchangeable by D_2O); Anal. calcd. for

$C_{17}H_{12}FN_5O_2$ (337.31): C, 60.53; H, 3.59; N, 20.76. Found: C, 60.58; H, 3.64; N, 20.39.

*2-(2-((1H-pyrrol-2-yl)methylene)hydrazinyl)-4-(4-fluorophenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**3g**)*

Brown crystals; (0.42 g, 1.30 mmol), yield: 64.61%; m.p.: > 350 °C; IR (cm^{-1}): 3313 (3NH), 3109 (N=CH + ArH), 2193 (C≡N), 1647 (C=O); ^{1}H-NMR (DMSO-d_6-300 MHz, ppm): 6.12 (d, 1H, ArH), 6.42 (d, 1H, ArH), 6.77-6.87 (t, 1H, ArH), 7.07-7.16 (m, 2H, ArH), 7.81–7.91 (m, 2H, ArH), 7.97 (s, 1H, N=CH), 10.47 (s, 1H, NH, exchangeable by D_2O), 11.23 (s, 1H, NH, exchangeable by D_2O), 11.67 (s, 1H, NH, exchangeable by D_2O); Anal. calcd. for $C_{16}H_{11}FN_6O$ (322.30): C, 59.63; H, 3.44; N, 26.08. Found: C, 59.80; H, 3.41; N, 26.31.

*4-(4-Fluorophenyl)-2-(2-(4-hydroxybenzylidene)hydrazinyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**3h**)*

Yellow crystals; (0.35 g, 1.0 mmol), yield: 50%; m.p.: 322–324 °C; IR (cm^{-1}): 3446–3286 (2NH,OH), 3032 (N=CH + ArH), 2216 (C≡N), 1653 (C=O); ^{1}H-NMR (DMSO-d_6-300 MHz, ppm): 6.80 (d, 2H, J = 8.7 Hz, ArH), 7.10 (d, 2H, J = 8.7 Hz, ArH), 7.52 (d, 2H, J = 8.7 Hz, ArH), 7.86 (d, 2H, J = 8.7 Hz, ArH), 8.08 (s, 1H, N = CH), 9.77 (s, 1H, OH, exchangeable by D_2O), 10.01 (s, 1H, NH, exchangeable by D_2O), 10.58 (s, 1H, NH, exchangeable by D_2O); Anal. calcd. for $C_{18}H_{12}FN_5O_2$ (349.32): C, 61.89; H, 3.46; N, 20.05. Found: C, 62.04; H, 3.44; N, 20.23.

*4-(4-Fluorophenyl)-2-(2-(3-hydroxy-4-methoxybenzylidene) hydrazinyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**3i**)*

Dark yellow crystals; (0.49 g, 1.29 mmol), yield: 64.47%; m.p.: 240–242 °C; IR (cm^{-1}): 3545–3261 (2NH, OH), 3001 (N=CH + ArH), 2906 (CH aliphatic), 2212 (C≡N), 1699 (C=O); ^{1}H-NMR (DMSO-d_6-300 MHz, ppm): 3.84 (s, 3H, OCH_3), 6.80 (d, 2H, ArH), 7.04–7.15 (m, 2H, ArH), 7.29 (s, 1H, ArH), 7.83–7.92 (m, 2H, ArH), 8.08 (s, 1H, N=CH), 9.37 (s, 1H, OH, exchangeable by D_2O), 9.55 (s, 1H, NH, exchangeable by D_2O), 10.55 (s, 1H, NH, exchangeable by D_2O); Anal. calcd. for $C_{19}H_{14}FN_5O_3$ (379.34): C, 60.16; H, 3.72; N, 18.46. Found: C, 60.39; H, 3.76; N, 18.73.

General procedure of synthesis of compounds (4a–e)

A mixture of compound **2** (0.5 g, 2 mmol), anhydrous potassium carbonate (0.552 g, 4 mmol) and 4-substituted benzoyl chloride (2.2 mmol) in dry benzene (15 mL) was heated under reflux for 10 h. The reaction mixture was filtered while hot. The residue was washed with water (15 mL), dried and crystallized from ethanol.

*N'-(5-Cyano-4-(4-fluorophenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)benzohydrazide (**4a**)*

Yellow crystals; (0.11 g, 0.314 mmol), yield: 15.7%; m.p.: 268–270 °C; IR (cm^{-1}): 3388, 3338 (3NH), 3100 (ArH), 2204 (C≡N), 1685 (C=O), 1637 (C=O); ^{1}H-NMR (DMSO-d_6-300 MHz, ppm): 7.28–7.47 (m, 2H, ArH), 7.49–7.59 (m, 5H, ArH), 7.85–7.92 (m, 3H, ArH + NH), 10.50 (br. s, 2H, 2NH, exchangeable by D_2O); MS (*m/z*): 349.00 (M^+, 5.90%), 350.00 (M + H, 3.00%), 351.00 (M + 2, 1.66%), 105.00 (100.00%). Anal. calcd. for $C_{18}H_{12}FN_5O_2$ (349.32): C, 61.89, H, 3.46, N, 20.05. Found: C, 61.96, H, 3.51, N, 20.17.

*N'-(5-Cyano-4-(4-fluorophenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)-4-fluorobenzohydrazide (**4b**)*

Dark yellow crystals; (0.15 g, 0.41 mmol), yield: 20.54%; m.p.: > 300 °C; IR (cm^{-1}): 3442, 3340 (3NH), 3020 (ArH), 2206 (C≡N), 1660 (C=O), 1631 (C=O); ^{1}H-NMR (DMSO-d_6-400 MHz, ppm): 7.35–7.39 (m, 4H, ArH), 7.99–8.02 (m, 5H, ArH + NH), 10.55 (s, 2H, 2NH, exchangeable by D_2O); MS (*m/z*): 367.00 (M^+, 34.26%), 368.00 (M + H, 27.78%), 149.00 (100.00%). Anal. calcd. for $C_{18}H_{11}F_2N_5O_2$ (367.31): C, 58.86; H, 3.02; N, 19.07. Found: C, 58.97; H, 3.00; N, 19.19.

*4-Chloro-N'-(5-cyano-4-(4-fluorophenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)benzohydrazide (**4c**)*

Yellow crystals; (0.23 g, 0.599 mmol), yield: 29.87%; m.p.: > 300 °C; IR (cm^{-1}): 3446, 3421 (3NH), 3070 (ArH), 2204 (C≡N), 1656 (C=O), 1639 (C=O); ^{1}H-NMR (DMSO-d_6-400 MHz, ppm): 7.44–7.63 (m, 4H, ArH), 7.74–7.97 (m, 4H, ArH), 8.41 (s, 1H, NH, exchangeable by D_2O), 10.55 (s, 1H, NH, exchangeable by D_2O), 10.63 (s, 1H, NH, exchangeable by D_2O); Anal. calcd. for $C_{18}H_{11}ClFN_5O_2$ (383.76): C, 56.33; H, 2.89; N, 18.25. Found: C, 56.57; H, 2.87; N, 18.38.

*N'-(5-Cyano-4-(4-fluorophenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)-4-methylbenzohydrazide (**4d**)*

Brown crystals; (0.18 g, 0.49 mmol), yield: 24.65%; m.p.: 220–222 °C; IR (cm^{-1}): 3419, 3226 (3NH), 3057 (ArH), 2918 (CH aliphatic), 2206 (C≡N), 1660 (C=O), 1630 (C=O); ^{1}H-NMR (DMSO-d_6-300 MHz, ppm): 2.38 (s, 3H, CH_3), 7.32 (d, 2H, J = 6.0 Hz, ArH), 7.50 (d, 2H, J = 6.0 Hz, ArH), 7.78–7.86 (m, 4H, ArH), 7.98 (s, 1H, NH, exchangeable by D_2O), 10.38 (s, 1H, NH, exchangeable by D_2O), 11.50 (s, 1H, NH, exchangeable by D_2O); Anal. calcd. for $C_{19}H_{14}FN_5O_2$ (363.35): C, 62.81, H, 3.88, N, 19.27. Found: C, 62.81, H, 3.88, N, 19.27.

*N′-(5-Cyano-4-(4-fluorophenyl)-6-oxo-1,6-dihydropyrimidin-2-yl)-4-methoxybenzo hydrazide (**4e**)*

Brown crystals; (0.31 g, 0.82 mmol), yield: 40.78%; m.p.: 296–298 °C; IR (cm^{-1}): 3446, 3419 (3NH), 3055 (ArH), 2208 (C≡N), 1656 (C=O), 1639 (C=O); ^{1}H-NMR (DMSO-d_6-400 MHz, ppm): 3.81 (s, 3H, OC$\underline{H}_3$), 6.79-7.44 (m, 4H, Ar$\underline{H}$), 7.70-7.82 (m, 4H, Ar$\underline{H}$), 7.94 (s, 1H, N$\underline{H}$, exchangeable by D_2O), 8.22 (s, 1H, N$\underline{H}$, exchangeable by D_2O), 8.80 (s, 1H, N$\underline{H}$, exchangeable by D_2O); MS (*m/z*): 379.00 (M^+, 9.92%), 380.00 (M + H, 6.53%), 57.00 (100.00%). Anal. calcd. for $C_{19}H_{14}FN_5O_3$ (379.34): C, 60.16; H, 3.72; N, 18.46. Found: C, 60.38; H, 3.79; N, 18.60.

General procedure of synthesis of compounds (5a–c)

A mixture of compound **2** (0.5 g, 2 mmol), anhydrous potassium carbonate (0.83 g, 6 mmol) and 2-bromo-4′-substitutedacetophenone (2 mmol) in dry benzene (15 mL) was heated under reflux for 24 h. The reaction mixture was filtered while hot. The residue was washed twice with water (20 mL), dried and crystallized from methanol.

*2-(2-(2-(4-Chlorophenyl)-2-oxoethyl)hydrazinyl)-4-(4-fluorophenyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**5a**)*

Brown crystals; (0.32 g, 0.81 mmol), yield: 40.5%; m.p.: > 300 °C; IR (cm^{-1}): 3388–3421 (3NH), 3086 (ArH), 2964 (CH aliphatic), 2208 (C≡N), 1647 (C=O), 1631 (C=O); ^{1}H-NMR (DMSO-d_6-300 MHz, ppm): 3.67 (s, 2H, C$\underline{H}_2$), 6.8 (s, 1H, N$\underline{H}$, exchangeable by D_2O), 7.30 (d, 2H, J = 8.4 Hz, Ar$\underline{H}$), 7.42 (d, 2H, J = 9.0 Hz, Ar$\underline{H}$), 7.62 (d, 2H, J = 9.0 Hz, Ar$\underline{H}$), 7.79 (d, 2H, J = 8.4 Hz, Ar$\underline{H}$), 8.60(s, 1H, N$\underline{H}$, exchangeable by D_2O), 10.15 (s, 1H, N$\underline{H}$, exchangeable by D_2O); MS (*m/z*): 397.05 (M^+, 3.17%), 399.37 (M + 2, 1.38%), 117.02 (100.00%). Anal. calcd. for $C_{19}H_{13}ClFN_5O_2$ (397.79): C, 57.37; H, 3.29; N, 17.61. Found: C, 57.58; H, 3.34; N, 17.89.

*4-(4-Fluorophenyl)-6-oxo-2-(2-(2-oxo-2-(p-tolyl)ethyl)hydrazinyl)-1,6-dihydropyrimidine-5-carbonitrile (**5b**)*

Reddish brown crystals; (0.23 g, 0.609 mmol), yield: 30.26%; m.p.: 260 °C (Decomposed); IR (cm^{-1}): 3471, 3462, 3419 (3NH), 3066 (ArH), 2924, 2856 (CH aliphatic), 2216 (C≡N), 1678 (C=O), 1639 (C=O); ^{1}H-NMR (DMSO-d_6-300 MHz, ppm): 2.32 (s, 3H, C$\underline{H}_3$), 3.87 (s, 2H, C$\underline{H}_2$), 4.20 (s, 1H, N$\underline{H}$, exchangeable by D_2O), 7.22–7.24 (m, 4H, ArH), 7.78–7.80 (m, 5H, Ar$\underline{H}$ + N$\underline{H}$), 8.30 (s, 1H, N$\underline{H}$, exchangeable by D_2O); MS (*m/z*): 377.16 (M^+, 4.68%), 378.12 (M + H, 2.43%), 90.08 (100.00%). Anal. calcd. for $C_{20}H_{16}FN_5O_2$ (377.37): C, 63.65; H, 4.27; N, 18.56. Found: C, 63.81; H, 4.30; N, 18.72.

*4-(4-Fluorophenyl)-2-(2-(2-(4-methoxyphenyl)-2-oxoethyl)hydrazinyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**5c**)*

Brownish red crystals; (0.20 g, 0.508 mmol), yield: 25.32%; m.p.: 300 °C (Decomposed); IR (cm^{-1}): 3545, 3462, 3419 (3NH), 3064 (ArH), 2920, 2837 (CH aliphatic), 2218 (C≡N), 1670 (C=O), 1637 (C=O); ^{1}H-NMR (DMSO-d_6-400 MHz, ppm): 3.78 (s, 3H, $OC\underline{H}_3$), 3.82 (s, 2H, $C\underline{H}_2$), 5.89 (s, 1H, N<u>H</u> exchangeable by D_2O), 6.79 (d, 2H, J = 8.0 Hz, Ar<u>H</u>), 7.40–7.46 (m, 2H, Ar<u>H</u>), 7.69–7.85 (m, 5H, Ar<u>H</u> +N<u>H</u>), 8.23 (s, 1H, N<u>H</u> exchangeable by D_2O); Anal. calcd. for $C_{20}H_{16}FN_5O_3$ (393.37): C, 61.07; H, 4.10; N, 17.80. Found: C, 61.28; H, 4.16; N, 18.04.

General procedure of synthesis of compounds (6, 7)

A mixture of compound **2** (0.5 g, 2 mmol), and either ethyl acetoacetate (0.38 mL, 3 mmol) or ethyl cyanoacetate (0.23 mL, 2 mmol) in glacial acetic acid (20 mL) was heated under reflux for 8 h. The reaction mixture was filtered while hot and the filtrate was left to cool; the formed precipitate was filtered, washed with water (15 mL), dried and crystallized from ethanol.

*4-(4-Fluorophenyl)-2-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**6**)*

Pale yellow crystals; (0.25 g, 0.803 mmol), yield: 40.3%; m.p.: 298–300 °C; IR (cm^{-1}): 3454 (NH), 3070 (ArH), 2902, 2848 (CH aliphatic), 2225 (C≡N), 1680 (C=O), 1643 (C=O); ^{1}H-NMR (DMSO-d_6-400 MHz, ppm): 2.27 (s, 3H, $C\underline{H}_3$), 2.51 (s, 2H, $C\underline{H}_2$ overlapped), 5.37 (s, 1H, N<u>H</u> exchangeable by D_2O), 7.47–7.49 (m, 2H, Ar<u>H</u>), 8.11–8.15 (m, 2H, Ar<u>H</u>); Anal. calcd. for $C_{15}H_{10}FN_5O_2$ (311.27): C, 57.88; H, 3.24; N, 22.50. Found: C, 58.12; H, 3.29; N, 22.81.

*4-(4-Fluorophenyl)-2-(5-imino-3-oxopyrazolidin-1-yl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**7**)*

Pale yellow crystals; (0.19 g, 0.608 mmol), yield: 30.6%; m.p.: 240–242 °C; IR (cm^{-1}): 3305–3275 (3NH), 3088 (ArH), 2931 (CH aliphatic), 2222 (C≡N), 1683 (C=O), 1635 (C=O); ^{1}H-NMR (DMSO-d_6-300 MHz, ppm): 1.91 (s, 2H, $C\underline{H}_2$), 6.76 (d, 2H, J = 9.0 Hz, ArH), 7.80 (d, 2H, J = 9.0 Hz, Ar<u>H</u>), 8.31 (s, 1H, N<u>H</u>, exchangeable by D_2O), 9.72 (s, 1H, N<u>H</u>, exchangeable by D_2O), 9.97 (s, 1H, N<u>H</u>, exchangeable by D_2O). MS (*m/z*): 312.00 (M^+, 82.89%), 313.00 (M + H, 81.85%), 200.00 (100.00%); Anal. calcd. for $C_{14}H_9FN_6O_2$ (312.26): C, 53.85; H, 2.91; N, 26.91. Found: C, 53.99; H, 2.89; N, 26.91.

Biological evaluation

In-vitro cytotoxicity assessment

Cytotoxic activity screening was performed at Al-Azhar University, Faculty of Pharmacy, Pharmacology Department. All compounds were evaluated for cytotoxic activity against MCF-7, HepG2, A549 and Caco-2 cells using XTT assay [24]. 5-FU was employed as positive control. Exponentially, cells were seeded at a density of 5×10^3 cells/well into 96-well plates. For each compound, a set of concentration range of 1–500 µg/mL was used. Cell viabilities were determined 72 h post-incubation using XTT assay. Each concentration was processed four times then the IC_{50} value was calculated. MCF-7, HepG2 and A549 cells were maintained in RPMI 1640 media supplemented with 10% fetal calf serum (FCS), 100 U/mL penicillin and 100 mg/mL streptomycin. Caco-2 cells were maintained in Dulbelcco's modified Eagle's medium (DMEM), supplemented with 10% FCS, 500 U/mL penicillin, 500 mg/mL streptomycin, 1% sodium pyruvate and 1% L-glutamine. All cells were cultivated at 37 C, 5% CO_2 and 95% humidity. The dye was obtained from Sigma-Aldrich (Munich, Germany).

Antimicrobial activity screening

The testing of the antimicrobial activity of all novel derivatives was carried out at the Department of Microbiology, Faculty of Pharmacy, Misr University for Science and Technology using the agar well diffusion technique according to Jahangirian et al. [25]. The tested strains included: Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC CC33) and Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and fungus *Candida albicans*. The diameters of the measured zones showing complete inhibition were recorded to the nearest millimeter.

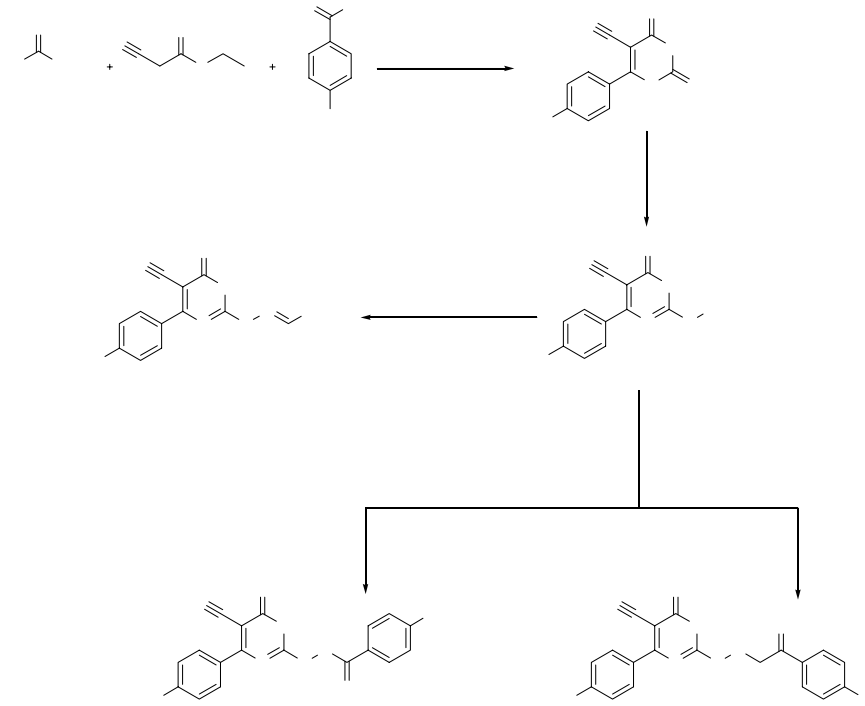
Determination of the minimum inhibitory concentration (MIC)

MIC values of the most active synthesized compounds were determined against *S. aureus* (ATCC 25923) and *B. subtilis* (ATCC CC33) using ciprofloxacin as reference drug according to microbroth dilution method [26].

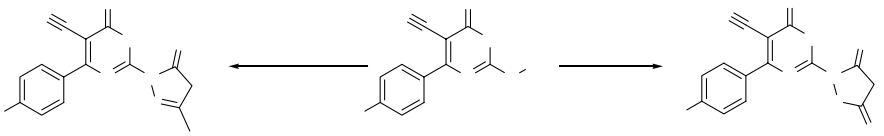
Results and discussion

Chemistry

The synthesis of new pyrimidinone-5-carbonitrile derivatives was achieved through Schemes 1 and 2. The structure of the newly synthesized compounds was confirmed by elemental analysis and spectral data (IR, ^{1}H-NMR, ^{13}C-NMR and MS). Numerous research works have focused on the synthesis of pyrimidinone derivatives [23,



Scheme 1 Reagents and conditions: (i) anhydrous K_2CO_3/absolute ethanol/reflux 5 h/48%; (ii) NH_2NH_2/reflux 12–14 h/58%; (iii) the appropriate aldehyde/glacial acetic acid/absolute ethanol/reflux 6 h/30–75%; (iv) 4-substituted benzoyl chloride/anhydrous K_2CO_3/dry benzene/reflux 10 h/15–40%; (v) 2-bromo-4′-substituted acetophenone/anhydrous K_2CO_3/dry benzene/reflux 24 h/25–40%



Scheme 2 Reagents and conditions: (i) $CH_3COCH_2COOCH_2CH_3$/glacial acetic acid/reflux 8 h/40%; (ii) $NCCH_2COOCH_2CH_3$/glacial acetic acid/reflux 8 h/30%

27, 28]. In this context, the starting pyrimidinone-5-carbonitrile **1** was synthesized in a one-pot, three-component reaction as outlined in Scheme 1, according to the reported procedure [23]. Furthermore, the synthesis of 4-(4-fluorophenyl)-2-hydrazinyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**2**) has already been described by the reaction of **1** with excess of hydrazine hydrate [19].

Preparation of new compounds **3a–i** was achieved following a previously reported procedure for analogous compounds [29–32], in which compound **2** was treated with the appropriate aldehyde in absolute ethanol in the presence of a catalytic amount of glacial acetic acid. The structures of **3a–i** were proven by the IR spectra, which revealed the disappearance of the forked peak of NH_2 in the key intermediate **2**. The ^{1}H-NMR spectra of compounds **3a-i** showed the appearance of a singlet signal of one proton around δ 7.97–8.17 ppm, pointing to the presence of CH=N and two exchangeable singlet signals around δ 9.55–12.50 ppm due to 2NH protons. Moreover, the ^{1}H-NMR spectra of compounds **3c** and **3f** showed the appearance of a singlet signal at δ 2.34 ppm with integration of three protons disclosing the presence of a methyl group. In addition, the ^{1}H-NMR spectra of compounds **3d** and **3i** indicated a singlet signal of three protons around δ 3.8 ppm disclosing the presence of a methoxy group.

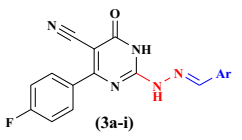
Alternatively, the hydrazinyl intermediate **2** was coupled with substituted benzoyl chloride or substituted phenacyl bromide in dry benzene using anhydrous potassium carbonate as an acid binder to afforded hydrazide derivatives **4a–e** and 2-(2-aryl-2-oxoethyl)hydrazinyl derivatives **5a–c**, respectively (Scheme 1). The IR spectra of compounds **4a–e** and **5a–c** are characterized by the appearance of two absorption bands around 1630–1685 cm^{-1}, disclosing the presence of two carbonyl groups. In addition, the ^{1}H-NMR spectra of compounds **4d** and **4e** displayed a singlet signal of three protons at δ 2.38 and 3.81 ppm, respectively corresponding to the methyl and methoxy groups. The ^{1}H-NMR spectra of compounds **5a–c** revealed the appearance of a singlet signal of two protons between δ 3.67-3.87 ppm corresponding to CH_2 of oxyethyl spacer. Additionally, the ^{1}H-NMR spectra of compounds **5b** and **5c** disclosed a singlet signal of three protons at δ 2.32 and 3.78 ppm, respectively pointing to the presence of methyl and methoxy groups.

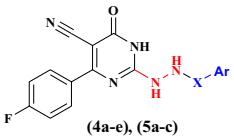
On the other hand, cyclocondensation of the hydrazinyl intermediate **2** with ethyl acetoacetae or ethyl cyanoacetate in glacial acetic acid furnished 2-(3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)pyrimidine-5-carbonitrile **6** and 2-(5-imino-3-oxopyrazolidin-1-yl)pyrimidine-5-carbonitrile **7**, respectively (Scheme 2). The IR spectra of compounds **6** and **7** showed two carbonyl stretch bands at 1643 and 1680 cm^{-1} for compound **6** and at 1635 and 1680 cm^{-1} for compound **7**. The ^{1}H-NMR spectrum of compound **6** revealed the appearance of a singlet signal of three protons at δ 2.27 ppm corresponding to the presence of a methyl group, and a singlet signal of two protons at δ 2.51 ppm disclosing the presence of CH_2 of pyrazole. The ^{1}H-NMR spectrum of compound **7** showed the presence of three exchangeable singlet signals each integrated for one proton at δ 8.31 and 9.72 and 9.97 ppm corresponding to three NH protons along with a singlet signal of two protons at δ 1.91 ppm assigned for the pyrazolidinone CH_2.

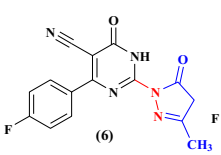
Cytotoxic activity

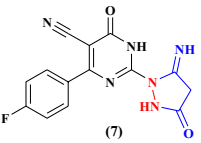
All the newly synthesized compounds were evaluated for their cytotoxic activity against four human cancer cell lines; namely MCF-7 (breast), HepG2 (liver), A549 (lung) and Caco-2 (colon), compared to 5-FU. The results are shown in Table 1 and

Table 1 IC_{50} in μM of 5-FU and the test substances against MCF-7, HepG2, A549 and Caco-2 cells









Compound no.	Ar	X	IC_{50} (μM[a])			
			MCF-7	HepG2	A549	Caco-2
5-FU	–	–	1.71 ± 0.10	4.12 ± 0.23	10.32 ± 0.61	20.22 ± 0.19
3a	$4\text{-FC}_6\text{H}_4$	–	42.19 ± 2.53	2.38 ± 0.09	> 100	29.75 ± 0.29
3b	$4\text{-ClC}_6\text{H}_4$	–	1.50 ± 0.07	92.31 ± 3.69	93.46 ± 6.95	17.04 ± 0.15
3c	$4\text{-CH}_3\text{C}_6\text{H}_4$	–	37.91 ± 1.90	2.35 ± 0.10	2.07 ± 0.16	29.97 ± 0.35
3d	$4\text{-OCH}_3\text{C}_6\text{H}_4$	–	39.09 ± 1.76	2.32 ± 0.10	> 100	29.22 ± 0.21
3e	$3,4,5\text{-(OCH}_3)_3\text{C}_6\text{H}_2$	–	1.53 ± 0.08	2.19 ± 0.12	99.91 ± 5.94	23.32 ± 0.14
3f	$5\text{-CH}_3\text{C}_4\text{H}_2\text{O}$	–	1.48 ± 0.09	92.31 ± 5.54	88.14 ± 6.17	16.15 ± 0.13
3g	$\text{C}_4\text{H}_4\text{N}$	–	1.42 ± 0.09	> 100	1.98 ± 0.16	9.50 ± 0.09
3h	$4\text{-OHC}_6\text{H}_4$	–	25.22 ± 0.90	3.46 ± 0.08	2.00 ± 0.14	22.07 ± 0.24
3i	$4\text{-OH-3-OCH}_3\text{C}_6\text{H}_3$	–	30.09 ± 1.02	2.31 ± 0.11	> 100	77.40 ± 1.54
4a	C_6H_5	C=O	1.58 ± 0.10	> 100	93.12 ± 7.44	26.08 ± 0.29
4b	$4\text{-FC}_6\text{H}_4$	C=O	49.90 ± 1.10	2.20 ± 0.12	92.60 ± 6.38	30.09 ± 0.37
4c	$4\text{-ClC}_6\text{H}_4$	C=O	1.61 ± 0.08	2.26 ± 0.12	> 100	37.95 ± 0.38
4d	$4\text{-CH}_3\text{C}_6\text{H}_4$	C=O	52.07 ± 3.12	2.28 ± 0.11	> 100	41.70 ± 0.50
4e	$4\text{-OCH}_3\text{C}_6\text{H}_4$	C=O	37.56 ± 2.10	2.24 ± 0.12	86.21 ± 6.29	30.08 ± 0.33
5a	$4\text{-ClC}_6\text{H}_4$	$\text{CH}_2\text{–C=O}$	1.52 ± 0.09	> 100	2.02 ± 0.16	23.77 ± 0.22
5b	$4\text{-CH}_3\text{C}_6\text{H}_4$	$\text{CH}_2\text{–C = O}$	1.62 ± 0.11	2.09 ± 0.15	75.88 ± 5.32	36.87 ± 0.51
5c	$4\text{-OCH}_3\text{C}_6\text{H}_4$	$\text{CH}_2\text{–C=O}$	1.54 ± 0.09	2.05 ± 0.16	88.14 ± 7.23	24.01 ± 0.30
6	–	–	60.07 ± 4.03	2.28 ± 0.12	2.00 ± 0.08	61.41 ± 1.10
7	–	–	59.94 ± 3.92	> 100	93.64 ± 7.40	60.10 ± 1.14

[a]The values given are mean ± SE of four experiments

represented graphically in Fig. 3. The results indicated that most of compounds possessed moderate to potent cytotoxic activities against one or more cell lines. In particular, MCF-7 and HepG2 were found to be more sensitive to the tested compounds than A549 and Caco-2.

Regarding the MCF-7 cell line, the hydrazone derivatives **3a–i** displayed potent to moderate cytotoxic activity (IC_{50} = 1.42–42.19 μM) and their potency was affected by the nature and type of substituent on the arylidine moiety. Compounds bearing a heterocyclic ring-like 5-methylfuran (**3f**) or pyrrole (**3g**) were the most potent compounds in this study (IC_{50} = 1.48 and 1.42 μM, respectively). The potency was also favored by grafting an electron-withdrawing substituent to the benzylidine-like 4-chlorine atom (**3b**, IC_{50} = 1.50 μM) or the *m*-methoxy groups (**3d**, IC_{50} = 1.53 μM). Meanwhile, an electron-donating substituent on the benzylidine

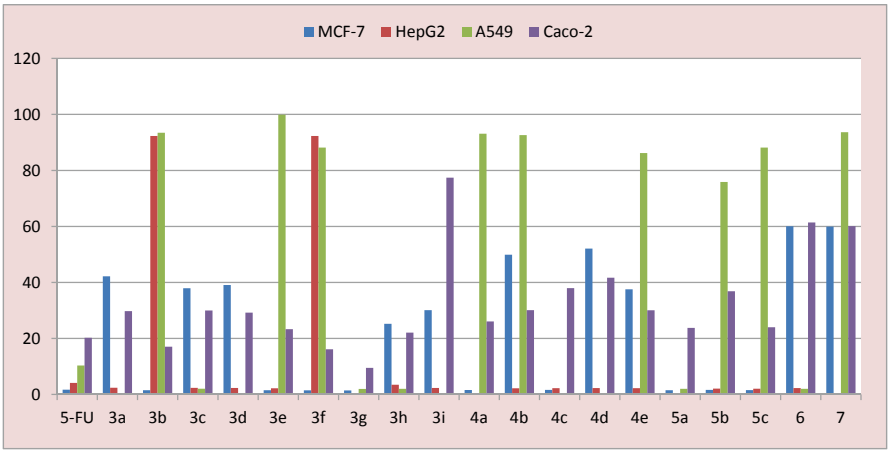


Fig. 3 IC_{50} in μM of 5-FU and the test substances against MCF-7, HepG2, A549 and Caco-2 cells

as 4-methyl (**3c**), 4-methoxy (**3d**) or 4-hydroxy (**3h** and **3i**) group resulted in compounds having moderate cytotoxic activity with an IC_{50} range = 25.22–39.09 μM. On the other hand, changing the methine linker in the hydrazone derivatives **3a–d** into C=O in **4b–e** produced compounds with similar level of cytotoxicity (compare compound **3a** ≠ **4b**, **3b** ≠ **4c**, **3c** ≠ **4d** and **3d** ≠ **4e**), whereas, replacing the methine linker in the hydrazone derivatives **3c** and **3d** (IC_{50} = 37.91 and 39.09 μM, respectively) by CH_2–C=O in **5b** and **5c** resulted in about a 25-fold increase in potency (IC_{50} = 1.62 and 1.54 μM, respectively). Furthermore, direct attachment of functionalized pyrazole or a pyrazolidine ring to the pyrimidinone-5-carbonitrile scaffold in **6** and **7** led to a mediocre cytotoxic effect.

The majority of the compounds showed potent cytotoxic activity against the HepG2 cell line with IC_{50} ranging from 2.05 to 3.46 μM. In contrast to the MCF-7 cell line, only compounds with heterocyclic moieties **3f**, **3g**, **7** and those with an (un)/4-chlorosubstituted terminal phenyl ring, compounds **4a**, **3b** and **5a**, exerted weak inhibitory effect against HepG2 (IC_{50} > 90 μM).

With respect to the less sensitive cell lines, five compounds, **3c**, **3g**, **3h**, **5a** and **6**, showed potent cytotoxic activity against A549 with IC_{50} values between 1.98 and 2.07 μM. While all the tested compounds exhibited moderate to weak anti-proliferative activity against Caco-2, only compounds **3b**, **3f** and **3g** were more potent than 5-FU (IC_{50} = 17.04, 16.15, 9.50 and 20.22 μM, respectively).

Antimicrobial activity

The new compounds were further evaluated for their antimicrobial activity against Gram-positive bacteria (*S. aureus* ATCC 25923 and *B. subtilis* ATCC CC33), Gram-negative bacteria (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853) and fungus (*Candida albican*). The diameters of the zones showing complete inhibition

of the microorganism growth were recorded to the nearest millimeter. The mean of the inhibition zone (IZ) is tabulated in Table 2.

The results revealed that, the target compounds had no significant activity toward Gram-negative bacteria and *Candida albican,* except for compounds **3f** and **3g**, which showed good activity against *E.coli* with an IZ of 19 and 17 mm, respectively. On the other hand, the majority of compounds possessed potent to good antibacterial activity against Gram-positive bacteria (*S. aureus* and *B. subtilis*). Compounds **3f**, **3g** and **6** showed the highest activity against *S. aureus* with IZ = 29, 25 and 22 mm, respectively, compared with 22 mm for the reference drug ciprofloxacin. Moreover compounds **3c**, **3e**, **4b** and **4c** demonstrated good activities against the same microorganism (IZ = 16–19 mm). As for *B. subtilis,* compounds **3f**, **3g**, **4b**, **4c**, **4d**, **5b** and **6** registered promising antibacterial activity (IZ rang = 21–17 mm) in comparison with ciprofloxacin (IZ = 24 mm). Accordingly, the quantitative assay of the antimicrobial activity was performed for the active compounds (**3c**, **3e**, **3f**, **3g**, **4b**, **4c** and **6**) with *S. aureus* and (**3f**, **3g**, **4b**, **4c**, **4d**, **5b** and **6**) with *B. subtilis* using ciprofloxacin as the reference drug in order to establish the MIC; the results are

Table 2 The preliminary antimicrobial and antifungal screening test for the prepared compounds using ciprofloxacin and fluconazole® as references, and DMSO as the control

Microorganism Compound no.	*S. aureus* (mm)	*B. subtilis* (mm)	*E. coli* (mm)	*P. aeruginosa* (mm)	*Candida albicans* (mm)
3a	12	12	–	–	
3b	–	–	–	–	–
3c	19	12	–	–	–
3d	–	–	–	–	
3e	17	–	–	–	
3f	29	19	19		–
3g	25	19	17		–
3 h	–	13	–	–	–
3i	–	15	–	–	–
4a	–	–	–	–	–
4b	18	20	–	–	–
4c	16	21	–	–	–
4d	–	17	–	–	–
4e	–	–	–	–	
5a	14	13	–	–	–
5b	14	17	–	–	–
5c	–	14	–	–	–
6	22	17			–
7	–	–	–	–	–
Ciprofloxacin	22	24	23	24	–
Fluconazole					28
DMSO	–	–	–	–	–

Table 3 Minimum inhibitory concentration (MIC) values in μg/mL of the tested compounds against *S. aureus* and *B. subtilis*

Compound no.	*S. aureus*	*B. subtilis*
3c	16	ND
3e	16	ND
3f	4	16
3g	8	16
4b	16	8
4c	32	32
4d	ND	32
5b	ND	32
6	8	32
Ciprofloxacin	2	1

ND not determined

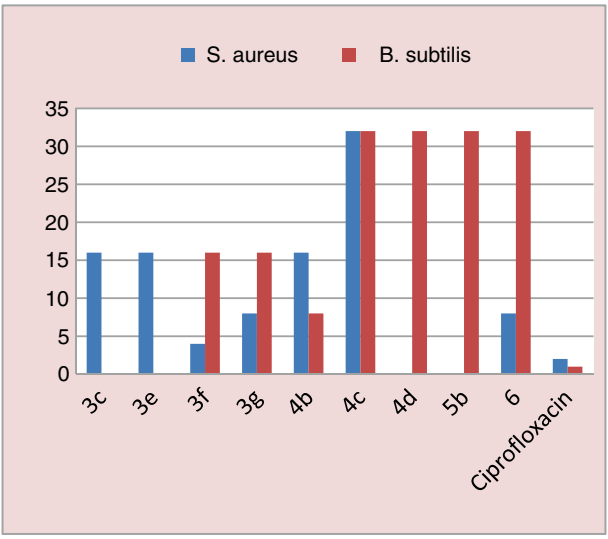


Fig. 4 Minimum inhibitory concentration (MIC) values (μg/mL) of the tested compound against *S. aureus* and *B. subtilis*

shown in Table 3 and Fig. 4. Analysis of the results revealed that compounds bearing terminal heterocyclic moieties such as **3f**, **3g** and **6** (MIC = 4, 8 and 8 μg/mL, respectively) were the most potent antibacterial agents against *S. aureus*, whereas the hydrazone derivatives with 4-methylbenzylidine **(3c)** and 4-methoxybenzylidine **(3e)** elicited reduced antibacterial effect (MIC = 16 μg/mL). On the other hand, the fluorobenzohydrazide derivative (**4b)** exhibited the highest inhibition effect toward *B. subtilis* (MIC = **8** μg/mL). Otherwise, the rest of the tested compounds were less active toward *B. subtilis* than *S. aureus* (MIC = 16–32 μg/mL).

Conclusion

The present study reports the synthesis of a novel series of pyrimidinone-5-carbonitriles **3a–i**, **4a–e**, **5a–c**, **6** and **7** as potential anticancer and antimicrobial agents. Several newly synthesized derivatives displayed potent cytotoxic activity against different cancer cells. Among the evaluated compounds, the hydrazone derivatives having a heterocyclic ring (**3f**, Ar = 5-methylfurane and **3g**, Ar = pyrrole) displayed a potent and broad spectrum of antitumor activity. Compound **3g** was the most potent on the MCF-7, A549 and Caco-2 cell lines with IC_{50} = 1.42, 1.98 and 9.50 μM, respectively, as compared with 5-FU (IC_{50} = 1.71, 10.32 and 20.22 μM, respectively), while compound **3f** was found especially effective against MCF-7 and Caco-2 cell lines with IC_{50} = 1.48 and 16.15 μM, respectively. Moreover the same compounds showed the highest antibacterial activity against the resistant G+*ve S. aureus* with promising inhibition effect toward *G+ve B. subtilis and G−ve E.coli*. In brief, compounds **3f** and **3g** represent good leads for optimization and design of more potent chemotherapeutic agents with anticancer and antimicrobial activities.

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Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

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